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Comparison of two different device results measuring HbA1c by high performance liquid chromatography (HPLC) method

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Abstract

Aim: HbA1c results, which are routinely measured in HA-8180T and HA-8180V model devices in our laboratory for HbA1c measurement, were divided into three groups based on diabetes mellitus diagnostic criteria with the aim of investigating the compatibility between the two devices at different HbA1c concentrations.

Material and Methods: For HbA1c analysis, the HbA1c values of 260 patients were measured in two devices (Arkray Adams HA-8180T and Arkray Adams HA-8180V) using the ion exchange chromatography method. According to the measured % HbA1c values, 3 groups (1st Group; n=87<5.7%, 2nd Group; n=96 5.7%- 6.4% and 3rd Group; n=77> 6.5%).

Results: Correlation (r=0.994, 95% Confidence Interval (CI) = 0.993-0.996, p<0.0001) of the measurement results obtained between the two devices and Passing-Bablok regression analysis [HA-8180T = 1.0xHA-8180V-0.20] (Slope 95% CI= 1.0-1.0, intercept 95% CI: -0.20-0.20) equation were obtained. According to the regression equation, the linearity between the devices was found to be (cusum test; p=0.90). In the Bland-Altman plot to evaluate the compatibility of the two devices, it was observed that the percentage change between the %HbA1c results obtained with HA-8180-T and the %HbA1c results obtained with HA-8180-V was 3% (95% CI: 2.83 – 3.12) higher on average.

Discussion: Due to the compatibility of the results measured between the two devices in this study, we think that the use of the HA-8180V device, which has a shorter result time, in laboratories with a higher number of tests may be appropriate in terms of reducing the workload.

Keywords

HbA1c, Device Comparison, HPLC, HA-8180 Analyzers

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Introduction

Hemoglobin is a metalloprotein found in erythrocytes, containing iron and having oxygen-carrying capacity. In the normal adult human, the hemoglobin molecule (HbA) accounts for about 97% of hemoglobin from two alpha and two beta chains (α2β2). The terms glycated hemoglobin, HbA1c (A1C test, A1C) are common expressions used to describe the glycation product formed by non-enzymatic binding of hemoglobin (Hb) A0 to the N-terminal (1-deoxyfructosyl) valine amino acid glucose [1].

HbA1c indicates an average blood glucose level of 2-3 months and is a marker used not only to guide the diagnosis and treatment of diabetes but also to assess the quality of patient care and predict the risk of developing diabetes complications [2, 3]. The fact that both intra-individual (CVI) and inter-individual (CVG) biological variation of HbA1c is lower than fasting plasma glucose and/or 2-hour plasma glucose (CVI and CVG values for HbA1c and plasma glucose are 1.2% and 5.0% and 4.8% and 8.1%, respectively), does not require pre-test preparation, is not affected by acute stress and has high preanalytic stability makes the HbA1c test advantageous [1-4]. Device changes and changes in measurement methods are quite common in laboratories. Studies show that there may be significant differences between HbA1c levels determined by different methods [4, 5]. Due to the importance of comparable results all over the world, the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) in 1995 and the National Glycohemoglobin Standardization Program (NGSP) in the United States the following year initiated standardization programs for HbA1c measurement. Many different measurement methods have been developed for HbA1c. These methods are based on charge difference (ion exchange chromatography, electrophoresis, capillary electrophoresis, isoelectric focusing) and structural difference (affinity chromatography, immunochemical analyses, enzymatic methods) [6-8]. HPLC (High-Performance Liquid Chromatography) is one of the most common analytical techniques used to measure HbA1C. In HPLC, ion exchange or affinity columns are used to distinguish HbA1C from other hemoglobin molecules [9].

HbA1C ≥ 6.5% or ≥48 mmol/mol measured using a method standardized according to the Diabetes Control and Complications Study (DCCT) and certified by the National Glychemoglobin Standardization Program (NGSSP) is one of the diagnostic criteria for diabetes mellitus according to the American Diabetes Association (ADA) criteria. The HbA1C analysis method used by all laboratories in the United States is required to be calibrated according to the high-performance liquid chromatography (HPLC) method, which is the gold standard for HbA1C [10, 11].

Depending on the technical features of different models of HPLC devices used in routine HbA1c measurements, such as the yield time (column elution time) and the ability to measure in variant mode, the number of tests run in the laboratory and the yield times may vary. In previous studies, method performance comparison studies of HA-8180T and HA-8180V (ADAMS Arkray) model devices belonging to the same manufacturer have been conducted and it has been reported that the compatibility between both devices is quite good [12, 13] . The HA-8180T detects HbA2, HbF, HbA1c (Stable HbA1c, S-A1c), HbS, HbC, HbE, and HbD variants with an analysis time of 210 seconds. The HA-8180V device is a reverse phase cation exchange chromatography device that provides HbA1c and HbF in 48 seconds in fast mode and HbA1c and HbF in 90 seconds in variant mode, as well as HbS, HbC, HbE and HbD detection.

In this study, HbA1c results, which are routinely measured in HA-8180T and HA-8180V model devices in our laboratory and perform HbA1c measurement, were divided into three groups based on diabetes mellitus diagnostic criteria, and the aim was to investigate the compatibility between the two devices at different HbA1c concentrations.

Material and Methods

Whole blood samples of 260 patients who applied to the laboratory of Ordu Training and Research Hospital for HbA1c analysis were taken into K3-EDTA tubes (VacutainerTM Becton-Dickinson, Rutherford, NJ, USA). This study was approved by the Clinical Researchers Ethics Committee of Ordu University (Date: 20.01.2023, No: 2023/27). For HbA1c analysis, the HbA1c values of 260 patients were measured in two devices using the ion exchange chromatography method (Arkray Adams HA-8180T and Arkray Adams HA-8180V, Japan). According to the HbA1c values of the patients, 3 groups (1st group <5.7%, 2nd group 5.7% - 6.4%, and 3rd group> 6.5%) were formed. No variant Hb was observed in any of the patients. Quality control procedures were applied for both devices throughout the study. Two levels (normal and pathological level) of the ICC sample (extendSURE ®) were used daily for internal quality control (ICC). The lot numbers of the controls were the same during the study period for HA-8180T (Lot no: 7125) and HA-8180V (Lot no: 7119).

Statistical Analysis

All statistical analyses were performed with the MedCalc (version 20.009; Ostend, Belgium) statistical package program. The conformity of the variables to the normal distribution was assessed using the Kolmogorov-Smirnov test. Values were given as mean, standard deviation (SD), median, and Q1-Q3. Regression analysis and Bland-Altman compatibility Graph were used to evaluate the compatibility of the results obtained in both devices with each other. The significant difference between the groups was evaluated at the level of $p<0.05$.

Results

HbA1c values of 260 patients measured in both devices are shown in Table 1. The % HbA1c values of the patient samples measured in both devices according to the groups are shown in Table 2. The correlation between the two devices (r=0.994, 95% Confidence Interval (CI) = 0.993-0.996, p<0.0001) and the equation [HA-8180T = 1.0xHA-8180V-0.20](Slope 95% CI = 1.0-1.0, intercept 95% CI : -0.20-0.20) were obtained in the Passing-Bablok regression analysis of 260 patients (Figure 1). According to the regression equation, the cusum test between the devices was found to be (p=0.90). In the Bland-Altman plot to evaluate the compatibility of the two devices, it was observed that the percentage change between the %HbA1c results obtained with HA-8180-T and the %HbA1c results obtained with HA-8180-V was higher on average by 3% (95% CI: 2.83 – 3.12) (Figure 2).

Discussion

Hemoglobin A1c is the main parameter for monitoring glycemic control in diabetic patients. In 2010, HbA1c was included in the ADA Diabetes Care Standards as a diagnostic criterion. The World Health Organization concluded in 2011 that HbA1c could be used as a diagnostic test [14]. Due to increased standardization, its use as a diagnostic criterion in diabetes is also increasing. Therefore, it is even more important that HbA1c measurement methods have sufficient diagnostic precision and accuracy and are comparable with other methods [15].

For the diagnosis of diabetes and effective treatment follow-up, HbA1c measurement must be reliable, reproducible, and highly accurate. However, results that do not reflect the correct value in HbA1c measurement may be obtained as a result of various factors that may interfere with the measurement, such as hemoglobinopathies, iron deficiency anemia, or vitamin B12 deficiency [16]. Due to the presence of Hb variants, high or low HbA1c results lead to errors in the diagnosis and treatment of diabetes. Identification of Hb variants during or before the HbA1c measurement process requires the selection of an HbA1c measurement method that is not affected by the variant or derivative in question. Thus, it is possible to measure the glycated Hb accurately [17].

Many methods have been developed for HbA1c measurement. These methods assess the charge (ion-exchange chromatography and electrophoresis) and structural difference (boronate affinity chromatography and immunological tests) between glycolyzed and non-glycolyzed hemoglobin species [18]. While there are studies indicating that there is a very good agreement between HPLC and the turbidimetric inhibition immunoassay (TINIA) method, [19, 20] there are also studies indicating that HbA1c values measured by the HPLC method are higher than TINIA [4, 21, 22]. The reason for this height in the HPLC measurement method is the interaction of the HbA1c peak with other contents and abnormal Hb variants. The HPLC (Ion exchange) method is based on the charge of the globin component of Hb. They stated that since abnormal Hb fragments are less positively charged than HbA, similar to glycosylated hemoglobin, their elution together from the column may affect the measurement results [21]. In another study conducted by Kın Tekçe et al., they stated that the HbA1c results obtained with the MQ-2000 PT device using the ion exchange chromatography method for HbA1c measurement were measured on average 0.37 higher than Architect C 8000 using the Tinia method, but this difference was within the limits predicted by NGSP [22]. In another study by Cihan et al., two different NGSP-approved HbA1c measurement methods (HPLC and Tinia) were compared. The HbA1c levels of the patients

Table 1. HbA1c % values measured in HA-8180T and HA-8180V

	HA-8180T	HA-8180V
N	260	260
Mean	6.46	6.27
SD	1.48	1.45
Median	6	5.8
Q1	5.5	5.4
Q3	6.8	6.6

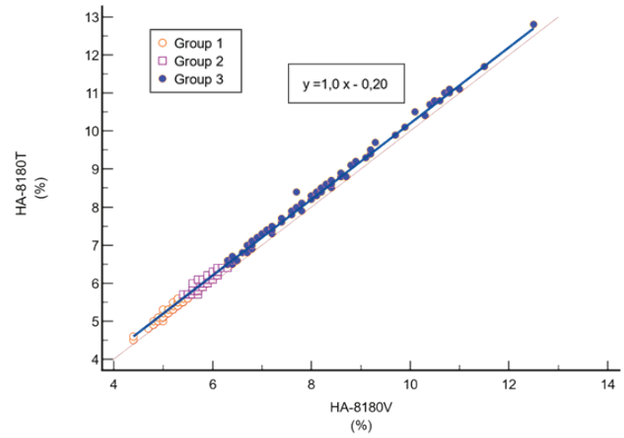

Figure 1. Passing-Bablok Regression equation of HA-8180T and HA-8180V

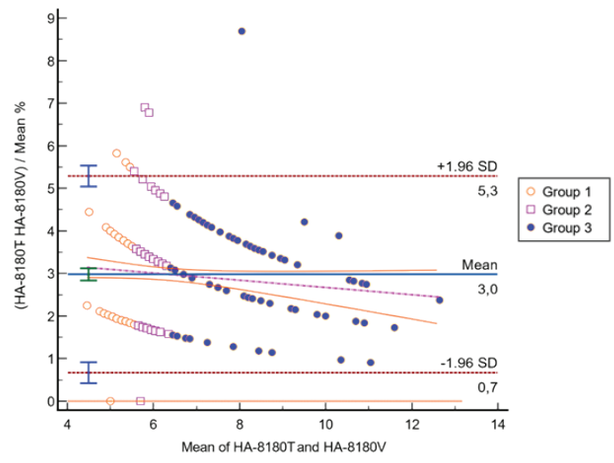

Figure 2. Bland-Altman graph of HA-8180-V and HA-8180-T devices

Table 2. Measurement results for each group of HA-8180T and HA-8180V devices

GROUPS	HA-8180T			HA-8180V		
	GROUP 1	GROUP 2	GROUP 3	GROUP 1	GROUP 2	GROUP 3
%HbA1c	< 5.7%	5.7- 6.4%	> 6.5%	< 5.7%	5.7- 6.4%	> 6.5%
N	87	96	77	87	96	77
Mean	5.34	6.00	8.29	5.18	5.82	8.5
SD	0.24	0.20	1.51	0.23	0.20	1.5
Median	5.4	6	8.1	5.2	5.8	7.8
Q1-Q3	5.2-5.5	5.8-6.1	7.09.2003	5.1-5.4	5.07.2006	6.8-9.1

were measured in three different hospitals as two devices using the HPLC method and a device using the TINIA method. At the end of the study, the mean HbA1c values measured by HPLC methods were found to be relatively higher than TINIA [4].

Similar to our study, in a study conducted by Urrechaga, the correlation between the results of the HA-8180T device and the HA-8180V device in the variant mode was found to be compatible, and the regression equation y=1,000x + 0.0 (Slope 95% CI 1,000-1,000; intercept 95% CI −0.00-0.00) was obtained [12]. In this study, in which we evaluated the effect of two different models of ARKRAY HPLC devices using the Ion Exchange Chromatography method on HbA1c values, we determined that the devices can be used interchangeably according to the passing-block regression equation [HA-8180T = 1.0xHA-8180V-0.20] (Slope 95% CI= 1.0-1.0, intercept 95% CI: -0.20 - 0.20). In addition, we did not observe any deviation from the linearity according to the equation (Cusum test p=0.90). In another study by Urrechaga, HA-8180T and HA-8190V devices were compared and the regression equation obtained was y= 1.022x -2.34 (Slope 95% CI 1.010-1.029; intercept 95% CI – 2.67-1.77). In the Bland-Altman compatibility graph, the difference in the averages of the devices was stated as 1.2 mmol/mol (0.11%)(13). In our study, in the Bland-Altman graph to evaluate the compatibility of both devices, we found that the HbA1c results obtained with HA-8180T were 3% higher on average (95% CI: 2.83 – 3.12) as a percentage change compared to the HbA1c results obtained with HA-8180V. However, especially in patients with high HbA1c concentration (for Group 3; > 6.5% HbA1c), we observed that the percentage change difference between the measurement results of the devices decreased and the consistency between the results increased.

The difference between the measurement methods should not exceed the level of clinical significance determined by the NGSP. The maximum permissible bias for HbA1c is 1.9% and the desired bias is 1.2% [23] . In this study, in which we compared both devices working with the HPLC method, the HA-8180V device was operated in fast mode with shorter results, and it was observed that there was an average of 3% bias between the devices. Although the HbA1c measurement method by HPLC method is the same for both devices, we think that the average percentage bias between the devices may occur due to the different elution times of the HbA1c fractions and the use of different calibrators of the devices. Since the aim of this study is not to compare the performance of the two devices by performing all the verification steps, the fact that all the verification studies of the devices could not be carried out constitutes the main limitation of our study.

Conclusion

In conclusion, in this study, we found that the HbA1c measurement results performed in the fast mode of the HA-8080V device in patients without hemoglobinopathy fit quite well compared to the HA-8080T device, which gives results in a longer time. We think that it may be appropriate to use the HA-8180V device in laboratories with a high number of tests, as it can reduce the workload of laboratories with a short time to give results.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and Human Rights Statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or compareable ethical standards.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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